DESCRIPTION OF PLATE 1.

Fig. 1.

Electrocardiogram, lead III.
Time intervals, 0·2 sec.
Record reads from left to right.

Fig. 2.

ECG. = Electrocardiogram, lead III.
H.W. = Hot Wire sphygmogram.
Rad = radial primary deflection.
Car = carotid primary deflection.
a = isolated auricular venous wave.
Dotted line = S-Radial interval.
Time intervals = 0·2 sec.
Record reads from right to left.

Fig. 3.

ECG. = Electrocardiogram, lead III.
H.W. = Hot Wire Radial Sphygmogram.
Time intervals = 0·2 sec.
Record reads from right to left.

Alterations in the Diameter of Erythrocytes during Hæmolysis.

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Introduction.

There has been some doubt as to the manner in which certain substances produce hæmolysis, especially saponin, bile salts, and those hæmolysins which are the result of immunity experiments. The doubt has even been extended to the mode of action of hypotonic saline, the hæmolytic action of which is generally put down to the osmotic changes which it produces. Bechhold (1), however, considers that surface tension phenomena have much to do with this hæmolysis. As regards saponin, some observers consider that it destroys the cell wall (Arrhenius (2)), whereas others consider that it produces hæmolysis by lowering of surface tension; others, again, deny that there is any relation between surface tension and hæmolytic activity in the case of the saponins (Woodward and Alsberg (3)). The same doubt prevails regarding bile salts, it being considered by many that they dissolve the lipoid envelope, and by others that surface tension plays an important part. As regards hæmolysis by hæmolytic amboceptors, it is suggested by some that the action is due to a sudden change in the permeability of the envelope (Clowes (4)), and by others that the lipoids are attacked (Jobling and Bull (5)); or, again, that "osmotic pressure is altered" by the action of the hæmolysin (McDonagh (6)).

Assuming what is generally admitted, that the red cell consists of an envelope containing a fluid or semi-fluid substance, some information may be gained by studying the changes of form which such a body would undergo under the various conditions of osmotic pressure, surface tension stress, or chemical action, and comparing with these the changes in form which may be observed in the red cell under the action of hæmolytic substances.

The erythrocyte, being circular on horizontal section and biconcave on vertical section, may be regarded, with very little error, as a large ellipsoid of rotation about its minor axis, having a semi-axis major = A and a semi-axis minor = B, from which two small ellipsoids, each equal to twice the volume of the concavity and having semi-axes a and b, are subtracted.

If to A, B, a, and b suitable values are assigned, the volume of the erythrocyte may be arrived at. Taking the usual average figures for the red cell:—

Since 2A, the diameter of the cell .... $= 7{\cdot}8\mu$
A, half the diameter of the cell .... $= 3{\cdot}9\mu$
The greatest thickness of the cell .... $= 2\mu$
And the least thickness of the cell .... $= 1{\cdot}5\mu$

$$\therefore\ 2b = 0{\cdot}5\mu \text{ and } b = 0{\cdot}25\mu.$$

$$\text{Further } 2\text{B} = \text{the least thickness} + 4b = 2{\cdot}5\mu,$$
$$\therefore\ \text{B} = 1{\cdot}25\mu.$$

The value of a may be taken as $= 2\mu$.

The volume of the cell is, then,

$$\frac{4\pi \text{A}^2\text{B}}{3} - 2\left[\frac{4\pi a^2 b}{3}\right]$$

and equals 71 cubic μ approximately. This agrees with the usually accepted volume.

Now an ellipsoid of rotation possessing an elastic wall will, if the internal pressure be raised, increase in volume in a definite way, tending to assume a form in which the pressure is equally distributed on its walls. Since the pressure is greatest where the curvature is least, the body will yield most at the extremities of the minor axis, and, by a lengthening of the minor axis, together with an initial decrease in the major axis, will eventually become a sphere, the radius of which will be less than the original major axis. If the pressure be further raised, the radius of the sphere will increase.

Applying this to the erythrocyte, it may be concluded that: I. If fluid pass into the cell by osmosis, the volume of the biconcave disc will increase by a bulging of the sides, the biconcavities disappearing. It is obvious that the cell will become an ellipsoid of volume $\frac{1}{3}(4\pi A^2B)$ without any stretching of the envelope. Taking average cells, the volume can increase in this way from about 70 cubic μ to about 80 cubic μ.

II. If diffusion continues B will increase, while A decreases slightly, until $B = A$, which is the condition for a sphere. Now the internal pressure will be equally distributed, and the volume of the cell will be $\frac{1}{3}(4\pi R^3)$, where R, the radius, $= A = B$. In the average cell again, this volume will be about 180 cubic μ.

III. If the volume continues to increase by continued diffusion into the cell, the value of R will increase until the cell becomes permeable to hæmoglobin.

It is, of course, to be understood that these considerations apply to the average cell, from which any individual cell may vary to some extent. It is possible to measure the variations which occur in the diameter of the cell, and consequently in the value of A, during hæmolysis by various agents.

A knowledge of these variations enables one to draw conclusions regarding the mode of action of the hæmolytic substance. For instance, if hæmolysis occurs because fluid passes into the cell by osmosis, distending it, and ultimately causing bursting, one may expect the value of A first to decrease, and then, when the cell assumes a spherical form, to increase until bursting occurs; the volume of the cell at various stages may be calculated, and thus it may be found how much distension the cell is capable of standing before the envelope gives way. If, on the other hand, hæmolysis occurs by chemical destruction of the cell wall, no change in the value of A will occur. These points will appear more clearly when examples are considered.

Method.

1. A 5 per cent. suspension in isotonic saline of the cells to be hæmolysed is prepared.

2. A drop of the suspension is placed on a hæmocytometer slide and covered; a number of fields are then photographed under a magnification of about 1200. Since each field usually contains about twenty cells, if five or six fields be photographed, sufficient cells for averaging purposes will be obtained. The diameters of these cells are measured from the negatives and the mean diameter found. The plates used should be as rapid as possible; using Wratten panchromatic plates, with a green light filter, which greatly improves the definition, an exposure of about 1 minute is sufficient, even "ghost corpuscles" being visible on the plate.

3. To a suitable volume of the suspension is added a suitable quantity of the hæmolytic agent, whose action is under investigation. The quantity to be

added is determined beforehand, so as to give a complete hæmolysis of the amount of suspension used in some convenient time, *e.g.*, 20 minutes. After various periods of time—1 minute, 5 minutes, 10 minutes, etc.—a drop of the mixture is placed on the slide, and, without delay, a photograph of a field is taken. Rapid working is essential. Since the number of cells seen in one such field is insufficient for purposes of calculation, the whole process is to be repeated five or six times, until plates are obtained showing about 100 cells at each of the periods of time arranged beforehand. If it is important to work at a definite temperature a warm stage is used. The diameters of the cells are measured from the negative, the mean diameter after 1 minute, 5 minutes, 10 minutes, etc., being thus found. Alterations in the diameter produced by the action of the hæmolytic substance, after various times, are obtained in this way; from such alterations several facts may be deduced.

Results will be given for hæmolysis by (1) hypotonic saline, (2) saponin, (3) sodium taurocholate, (4) a complement amboceptor system for sheep cells.

I. *Hæmolysis by Hypotonic Saline.*

Using a 5 per cent. suspension of human erythrocytes, the mean diameters in μ, their probable errors, and the standard deviations of the groups, are given in tabular form:—

Hæmolytic agent.	Time of action.	Mean diameter.	σ.
Controls		8·65 ± 0·0267	0·49
0·4 per cent. saline	3 minutes	7·4 ± 0·0262	0·45
0·4 „ „	7 „	8·3 ± 0·0390	0·55
0·4 „ „	20 „	9·0 ± 0·0330	0·59
0·4 „ „	30 „	9·5 ± 0·0345	0·62
0·3 „ „	1 minute	7·1 ± 0·0216	0·45
0·3 „ „	3 minutes	7·7 ± 0·0162	0·34
0·3 „ „	15 „	9·5 ± 0·0360	0·64

From these figures it may be concluded that the red cell, when undergoing distension due to fluid passing into it by osmosis, behaves as might be expected—the diameter decreases at the same time as the minor axis increases, until a sphere is formed; the radius of this sphere then increases.

The diameter of the average cell before hæmolysis began being 8·65 μ, its volume may be taken as 88·5 μ^3. Such a cell could attain a volume of 97·9 μ^3 without the capsule being stretched. From this point, as fluid continues to pass in, the diameter decreases until it becomes about 7·1 μ; thereafter it again increases, reaching in the final stages 9·5 μ, and having a volume of 448 μ^3. Red cells when hæmolysed by hypotonic saline, therefore, behave as ellipsoids under internal pressure, as would be expected. Before the capsule gives way, the average cell can increase to about five times its original volume.

II. *Hæmolysis by Saponin.*

A 5 per cent. suspension of human erythrocytes was used. The results are expressed as in (1). The concentration of saponin used was 1 in 20,000, in isotonic saline. Measurements are in μ.

Time of action.	Mean diameter.	σ.
Controls	8·65±0·0267	0·49
1 minute	5·8 ±0·0150	0·32
3 minutes	5·9 ±0·0138	0·25
5 ,, ...	6·0 ±0·0150	0·32
7 ,, ...	6·3 ±0·0240	0·45
9 ,, ...	6·3 ±0·0300	0·55
14 ,, ...	6·3 ±0·0300	0·55
25 ,, ...	6·3 ±0·0285	0·49

Owing to its property of lowering surface tension, saponin causes the red cell to become a sphere whose volume is the same as the volume of the cell before the saponin affected it. The radius of this sphere then gradually increases, its volume increasing by the passage of fluid from without. The increase is however small, for complete hæmolysis occurs when the average cell has a diameter of 6·3 μ, and a volume of only $131\ \mu^3$. The envelope is, therefore, not ruptured owing to distension of the cell alone, for it has been seen in (1) that it can withstand a much greater distension than this—but gives way sooner owing to its being weaker; the saponin having affected the membrane so that it can stand less stress.

III. *Hæmolysis by Sodium Taurocholate.*

A 5 per cent. suspension of human erythrocytes was used. The concentration of sodium taurocholate used was 1 in 2500 in isotonic saline. Measurements in μ.

Time of action.	Mean diameter.	σ.
Controls	8·15±0·0530	0·94
2 minutes	5·5 ±0·0180	0·32
5 ,, ...	6·0 ±0·0180	0·32
15 ,, ...	6·3 ±0·0450	0·80
20 ,, ...	6·3 ±0·0710	1·20
25 ,, ...	6·5 ±0·0540	0·96
45 ,, ...	7·0 ±0·0475	0·77
60 ,, ...	7·1 ±0·0330	0·59

The action of sodium taurocholate is similar to the action of saponin. Owing to the lowering of surface tension caused, the red cell, without alteration of volume, becomes a sphere. The radius of this sphere then increases, since there is an outward stress on the envelope, caused by the lowered surface tension. In order that this increase in the sphere may occur, fluid must pass into the cell; the rate at which this occurs determines the rate of increase in volume. Ultimately the average cell reaches a diameter of 7·1 μ which gives a volume of about 187 μ^3. The causes of rupture are again two—the distension of the cell, and the weakening of the envelope by chemical action. Since the cell is able to attain a larger volume before rupture of the envelope occurs than is the case with saponin hæmolysis, it may be concluded that sodium taurocholate weakens the envelope to a less extent than does saponin. The fact that the increase in volume of the cell, and accordingly the stretching of the envelope, under the surface tension stress, depends on the rate at which fluid can pass into the cell, and therefore on the permeability of the envelope to the surrounding fluid, explains the fact noted by Furhata (7) and Handovsky (8), that saponin hæmolysis is slow if the cells be suspended in a viscid fluid, or one which diffuses with difficulty, and also that salts which render the envelope less permeable, such as calcium, delay saponin hæmolysis, as observed by Horber (9).

IV. *Hæmolysis by Amboceptor-complement System.*

A 3 per cent. suspension of sheep cells was used, and hæmolysed by a hæmolytic serum for sheep cells, plus guinea-pig complement. Hæmolytic serum and complement were titrated beforehand so as to give a complete hæmolysis in 30 minutes. The experiment was conducted at 37° C. on a warm stage. Measurements in μ.

Time of action.	Mean diameter.	σ.
Controls	5·4±0·0200	0·31
5 minutes	5·3±0·0130	0·24
10 "	5·4±0·0185	0·32
20 "	5·4±0·0300	0·55
30 "	5·4±0·0228	0·42

During the time of action of the hæmolysin, there is no change in the mean diameter. It may be concluded, therefore, that the hæmolysis is produced by chemical action on the envelope, unaccompanied by osmotic or surface tension changes.

Summary.

The results obtained are, in general, such as might be expected. It may be concluded that:—

(1) The observation of alterations in the average diameter of numbers of erythrocytes undergoing hæmolysis by various hæmolytic agents, throws light on the mode of action of these substances.

(2) The changes in the diameter, in the case of hæmolysis by hypotonic saline, are those which might be anticipated, if the red cell be considered as an ellipsoid with an elastic wall and with fluid contents, subjected to pressure through the entering of fluid by osmosis.

(3) Saponin and sodium taurocholate afforded examples of substances which act by (*a*) producing a lowering of surface tension, and (*b*) by attacking the envelope; saponin acting on the envelope to a greater degree than the bile salt.

(4) The hæmolysis produced by a hæmolysin, plus complement, is produced by the envelope being weakened by chemical action; no observable surface tension or osmotic change accompanying the hæmolysis.

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